# Biotechnology & Industrial Microbiology

Microbiology & Immunology Dept.
Lecture 3

# Stages of Fermentation process

- Industrial fermentations comprise both **upstream processing** (USP) and **downstream processing** (DSP) stages.

# Stages of Fermentation process

**I) USP** involves all factors and processes leading to, and including, the fermentation, and consists of three main areas.

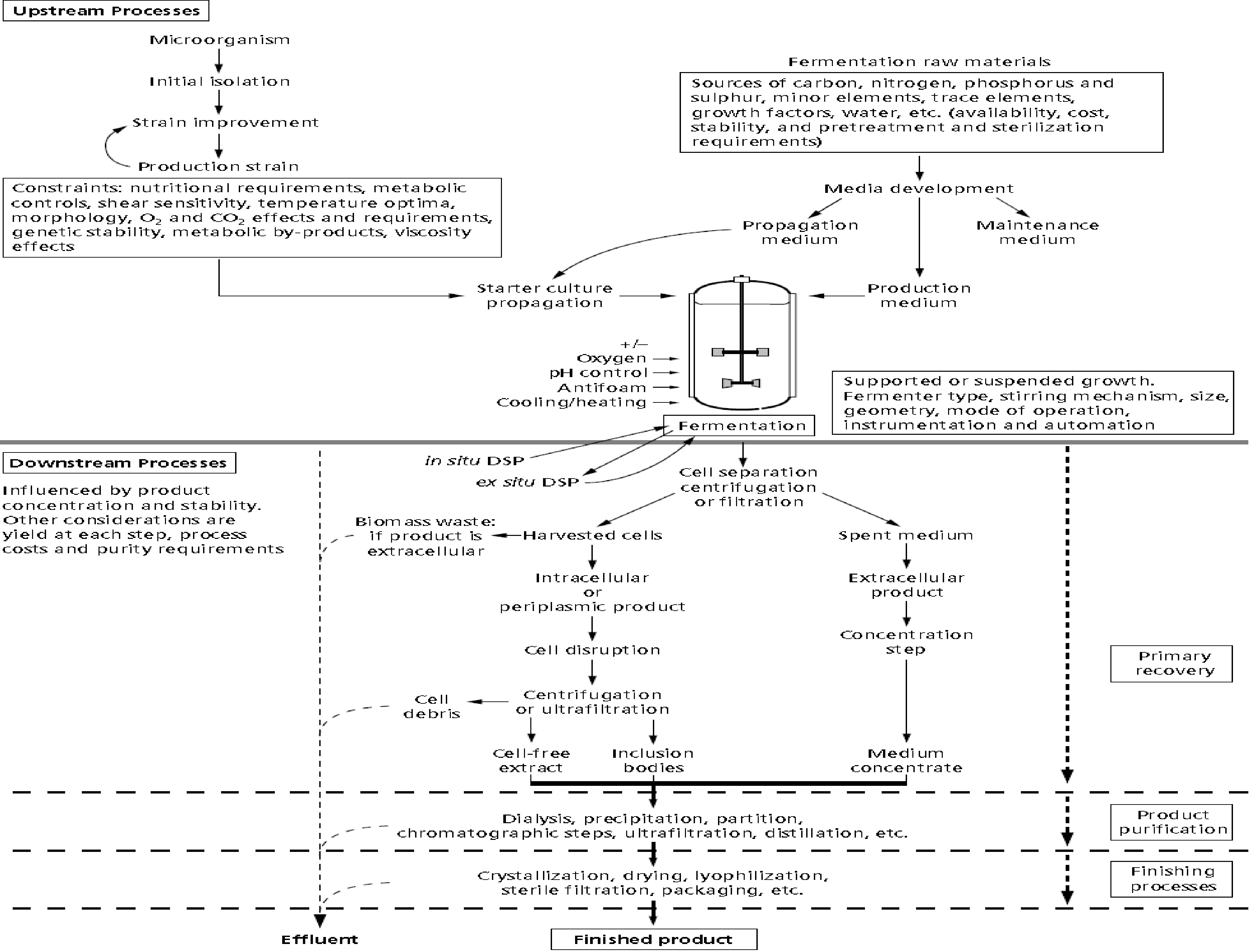
- The first relates to aspects associated with the **producer microorganism**. They include the strategy for initially obtaining a suitable microorganism, industrial strain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a suitable inoculums and the continuing development of selected strains to increase the economic efficiency of the process.

- The second aspect of USP involves **fermentation media**, especially the selection of suitable cost-effective carbon and energy sources, along with other essential nutrients. This media optimization is a vital aspect of process development to ensure maximization of yield and profit.
- The third component of USP relates to the **fermentation process**, which is usually performed under rigorously controlled conditions, developed to optimize the growth of the organism or the production of a target microbial product.

# Stages of Fermentation process

**II) DSP** encompasses all processes following the fermentation as (primary recovery, product purification, finishing process).

- It has the primary aim of efficiently, reproducibly and safely recovering the target product to the required specifications (biological activity, purity, etc.), while maximizing recovery yield and minimizing costs.
- It includes cell harvesting, cell disruption and product purification.


Upstream Processes
Microorganism
Initial isolation
Strain improvement
Production strain
Constraints: nutritional requirements, metabolic controls, shear sensitivity, temperature optima, morphology, $O_2$ and $CO_2$ effects and requirements, genetic stability, metabolic by-products, viscosity effects
Fermentation raw materials
Sources of carbon, nitrogen, phosphorus and sulphur, minor elements, trace elements, growth factors, water, etc. (availability, cost, stability, and pretreatment and sterilization requirements)
Media development
Propagation medium
Maintenance medium
Starter culture propagation
Production medium
+/−
Oxygen
pH control
Antifoam
Cooling/heating
Fermentation
Supported or suspended growth. Fermenter type, stirring mechanism, size, geometry, mode of operation, instrumentation and automation
Downstream Processes
Influenced by product concentration and stability. Other considerations are yield at each step, process costs and purity requirements
*in situ* DSP
*ex situ* DSP
Cell separation centrifugation or filtration
Biomass waste: if product is extracellular
Harvested cells
Spent medium
Intracellular or periplasmic product
Extracellular product
Cell disruption
Concentration step
Primary recovery
Cell debris
Centrifugation or ultrafiltration
Cell-free extract
Inclusion bodies
Medium concentrate
Dialysis, precipitation, partition, chromatographic steps, ultrafiltration, distillation, etc.
Product purification
Crystallization, drying, lyophilization, sterile filtration, packaging, etc.
Finishing processes
Effluent
Finished product

# Products of fermentation processes

I) Microbial cells (biomass): Baker's yeast, Mushroom.

II) Microbial enzymes: Catalase, amylase, protease, ….

III) Microbial metabolites:

-Primary metabolites : Ethanol, citric acid.

-Secondary metabolites: Antibiotics, health care products.

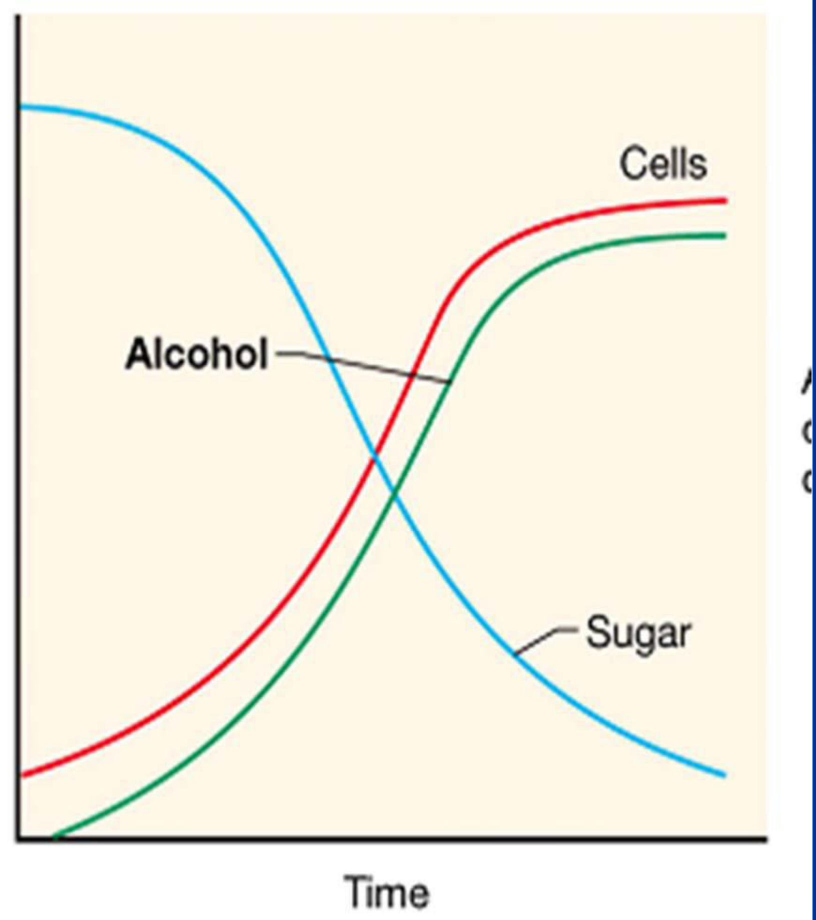
# Microbial metabolites

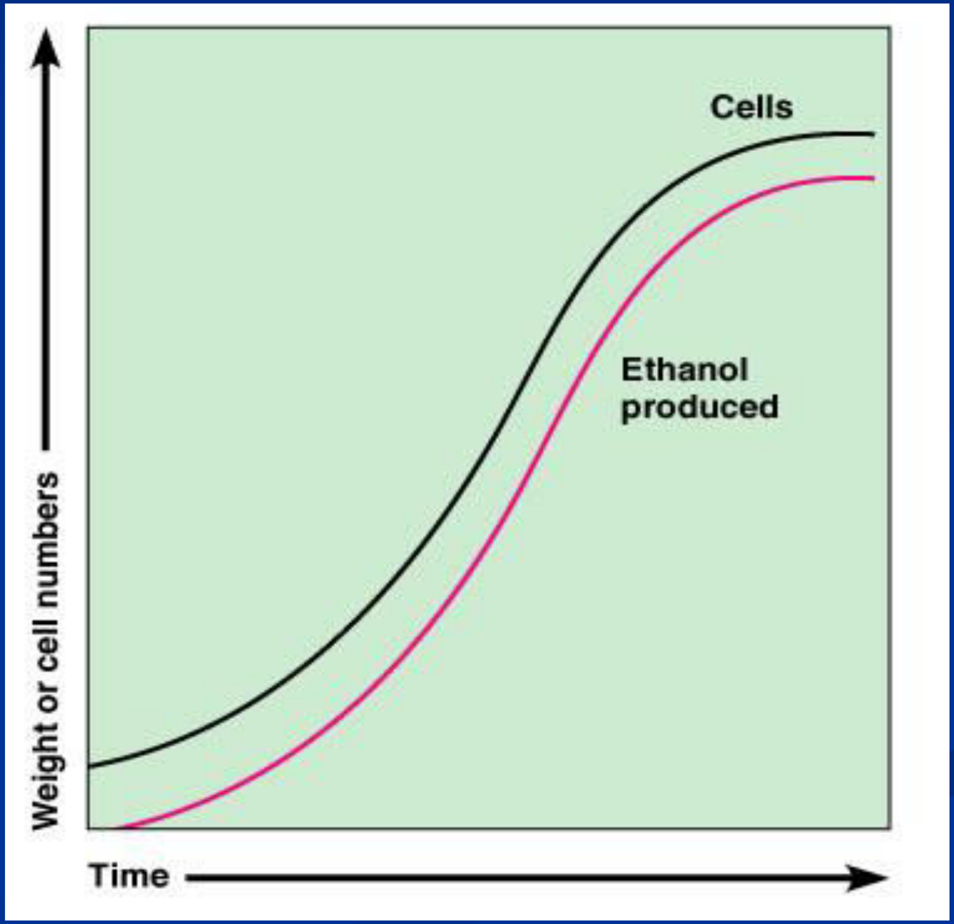
- **Primary metabolites:** are involved in growth, development, and reproduction of the organism. Thus it is a key component in maintaining normal physiological processes thus, it is often referred to as a central metabolite.

- Intermediates or end products of the primary metabolic pathways.

- Primary metabolites are typically formed during the **Log phase** of bacterial growth and are essential for proper growth.

Examples of primary metabolites include alcohols such as ethanol, lactic acid, vitamins and certain amino acids.



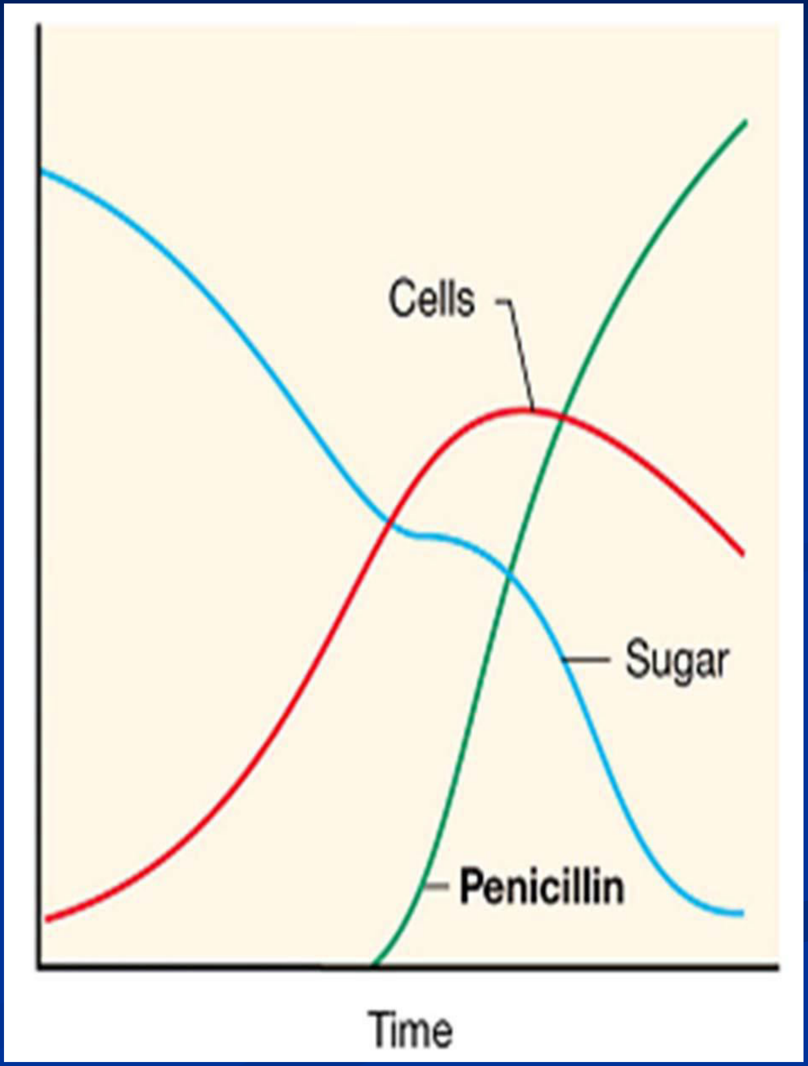
- **Secondary metabolites:** are typically organic compounds produced through the modification of primary metabolite synthases.

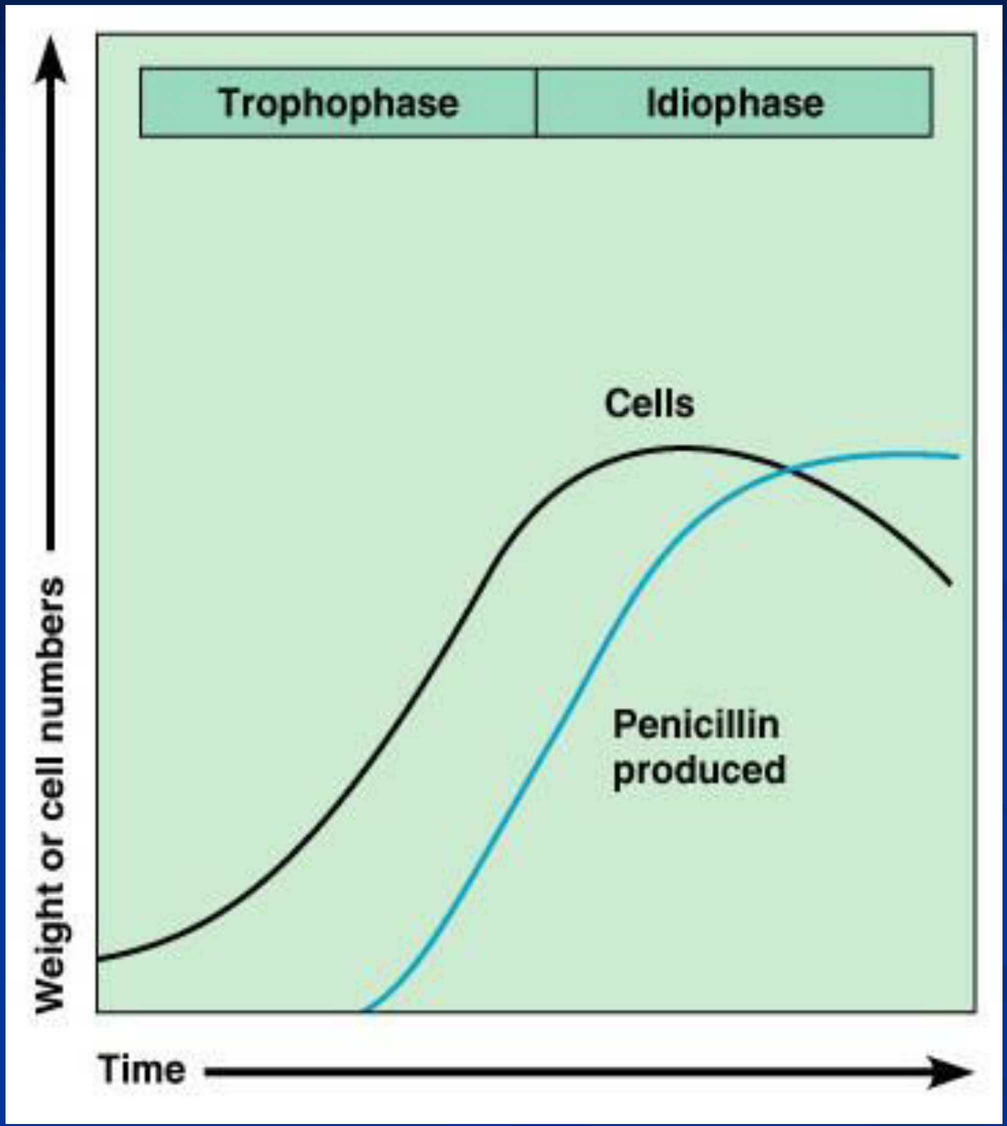
- Secondary metabolites do not play a role in growth, development, and reproduction like primary metabolites do. Not central metabolites.

- Many of the identified secondary metabolites have a role in ecological function, including defense mechanism (s), by serving as antibiotics and by producing pigments.

- Secondary metabolites are typically formed near the **stationary phase of growth**.

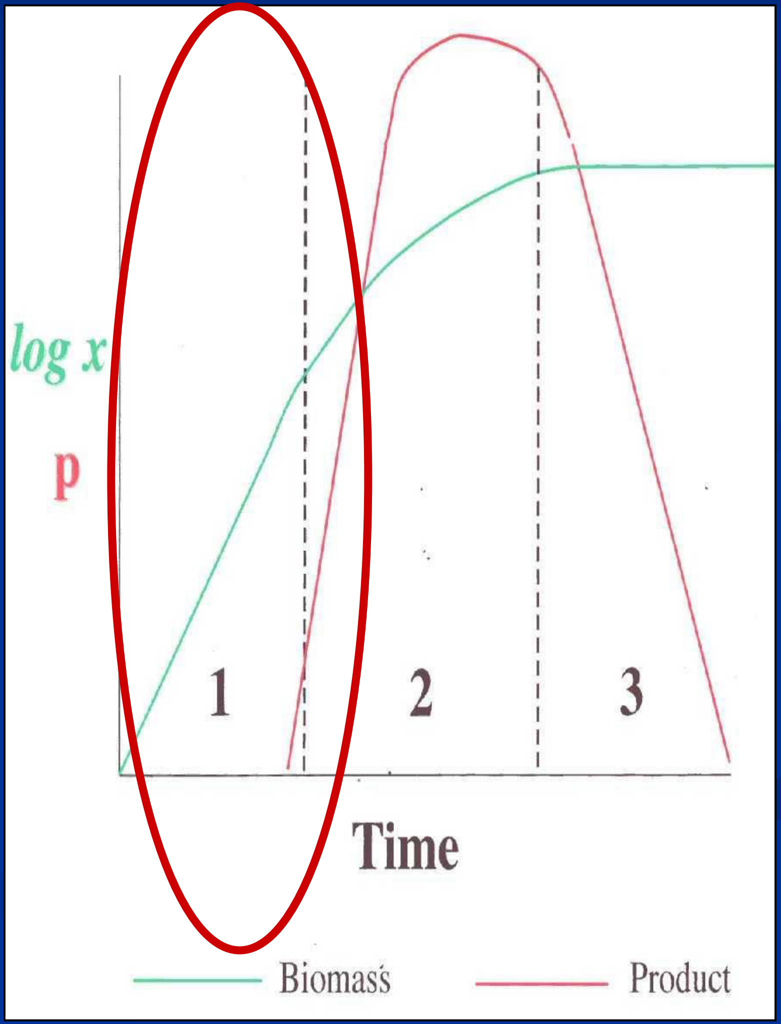
- Secondary metabolites are produced when the cell is not operating under optimum conditions e.g. when primary nutrient source is depleted.




Trophophase
Idiophase
Cells
Penicillin
produced
Weight or cell numbers
Time

# Phases of secondary metabolite production

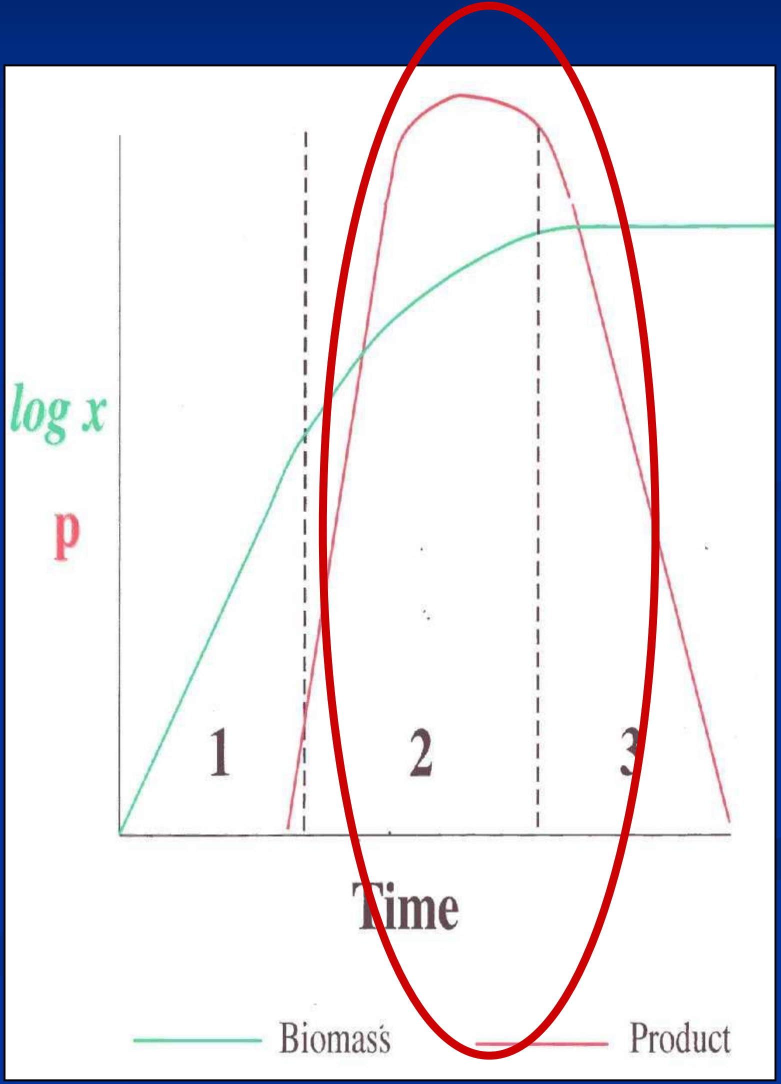
1. **Trophophase**
   - Culture is nutrient sufficient
   - Exponential Growth (Acceleration phase)
   - No Product Formation



# Phases of secondary metabolite production

2. **Idiophase**

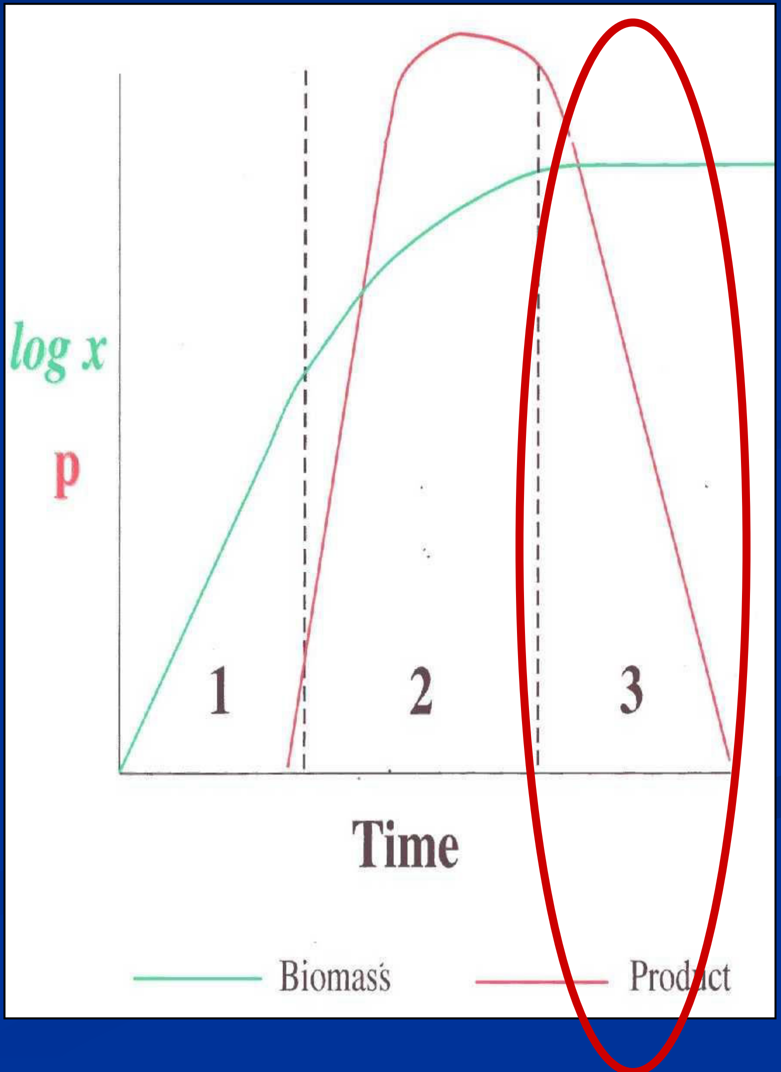
- Carbon limitation
- Growth slowing or stopped (Deceleration phase)
- Product formation
- HARVEST AT THE END OF THIS PHASE



# Phases of secondary metabolite production

## 3. Senescence

- Product formation ceases.
- Degeneration/lysis of mycelium (Fungi, Actinomycetes).
- Product degraded/used by culture.
- Microorganisms able to sporulate during this phase (form spores) for survival.



# Examples of important products produced by fermentation

# I. Microbial biomass production

- In biomass production, the cells produced during the fermentation process are the products.
- Consequently, the fermentation is optimized for the production of a maximum concentration of microbial cells.
- Microbial biomass is broadly used for three purposes:

1- Viable microbial cells are prepared as fermentation starter cultures and inocula for food and beverage fermentations, waste treatment processes, agricultural inoculants, and as biopesticides.

2- As a source of protein for human food.

3- Animal fodder.

**Ex1. Production of mushroom.**

**Ex2. Manufacture of baker's yeast:**

- A major fermentation industry has developed to manufacture baker's yeast required for making bread and associated bakery products.
- Two main types of baker's yeast are produced, compressed (cream) yeast and dry yeast.


# Production Process Description

- Baker's yeast production commences with propagation of a starter culture, which originates from a pure freeze-dried sample.
- Yeast cells are initially transferred to small liquid culture flasks, then on to larger intermediate vessels before being finally used to inoculate the large production fermenters of 50-350 m3 capacity. Overall, this may involve up to **eight scale-up stages** to produce the necessary final inoculum volume.

- The yeast is recovered from the final fermenter (50-350 m3) by using **centrifugal seperators** to remove the yeast from the fermentation broth.
- Harvested cells are then **washed** several times with water, and **dried** to around 70–75% (w/w) moisture using **vacuum filter dehydrators**. The yeast is usually packed in 1kg blocks and kept under refrigeration.

- Alternatively, the yeast may be dried further to 7–10% (w/w) moisture to form dried yeast, which can then be stored for long periods without refrigeration.

- Overall, the cycle of operations, from the initial pure yeast sample to the yeast blocks ready for sale, takes about **2 weeks**

# Medium composition

| Nutrient | Source |
|---|---|
| organism | *Saccharomyces cerevisiae* |
| carbon and energy source | Molasses |
| amino acids | biotin and pantothenic acid |
| nitrogen sources | ammonium salts or urea |
| minerals | orthophosphate and other mineral ions |
| pH | 4.0–4.4 |
| Aeration | Aerobic fermentation with an oxygen transfer rate of 150 mmol/L/h. (Ys: 0.54 g/g ) Maintained for 30 minutes after process |
| | anaerobic conditions (Ys: 0.12 g/g) |
| Operating modes | Submersion fed-batch process |

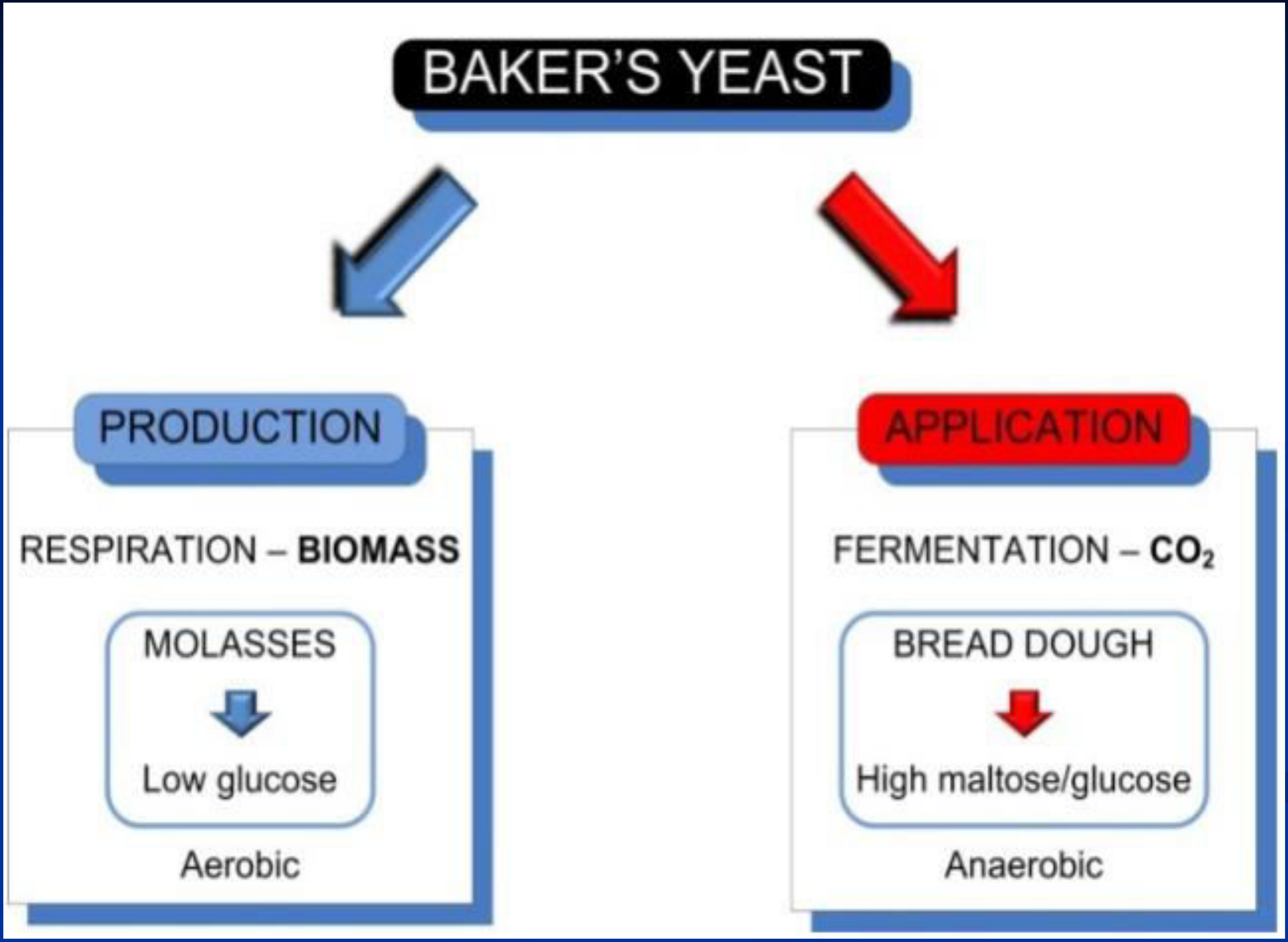
## Functions of yeast in baking:

### 1) Production of carbon dioxide:

Carbon dioxide is generated by the yeast as a result of the breakdown of fermentable sugars in the dough. The evolution of carbon dioxide causes expansion of the dough as it is trapped within the protein matrix of the dough.

### 2) Causes dough maturation:

This is accomplished by the chemical reaction of yeast produced alcohols and acids on protein of the flour and by the physical stretching of the protein by carbon dioxide gas. This results in the light, airy physical structure associated with yeast leavened products.


BAKER'S YEAST
PRODUCTION
RESPIRATION – BIOMASS
MOLASSES
Low glucose
Aerobic
APPLICATION
FERMENTATION – $CO_2$
BREAD DOUGH
High maltose/glucose
Anaerobic

# ***II. Microbial enzymes***

- Several thousand tones of commercial enzymes are currently produced each year, which have a value in excess of $1500 million.
- A few animal and plant enzymes are used, but most commercial enzymes are now obtained from microbial sources.
- Most industrial enzymes are products of **batch processes** and few are currently produced via continuous fermentation
- Bulk microbial enzymes versus fine microbial enzymes??

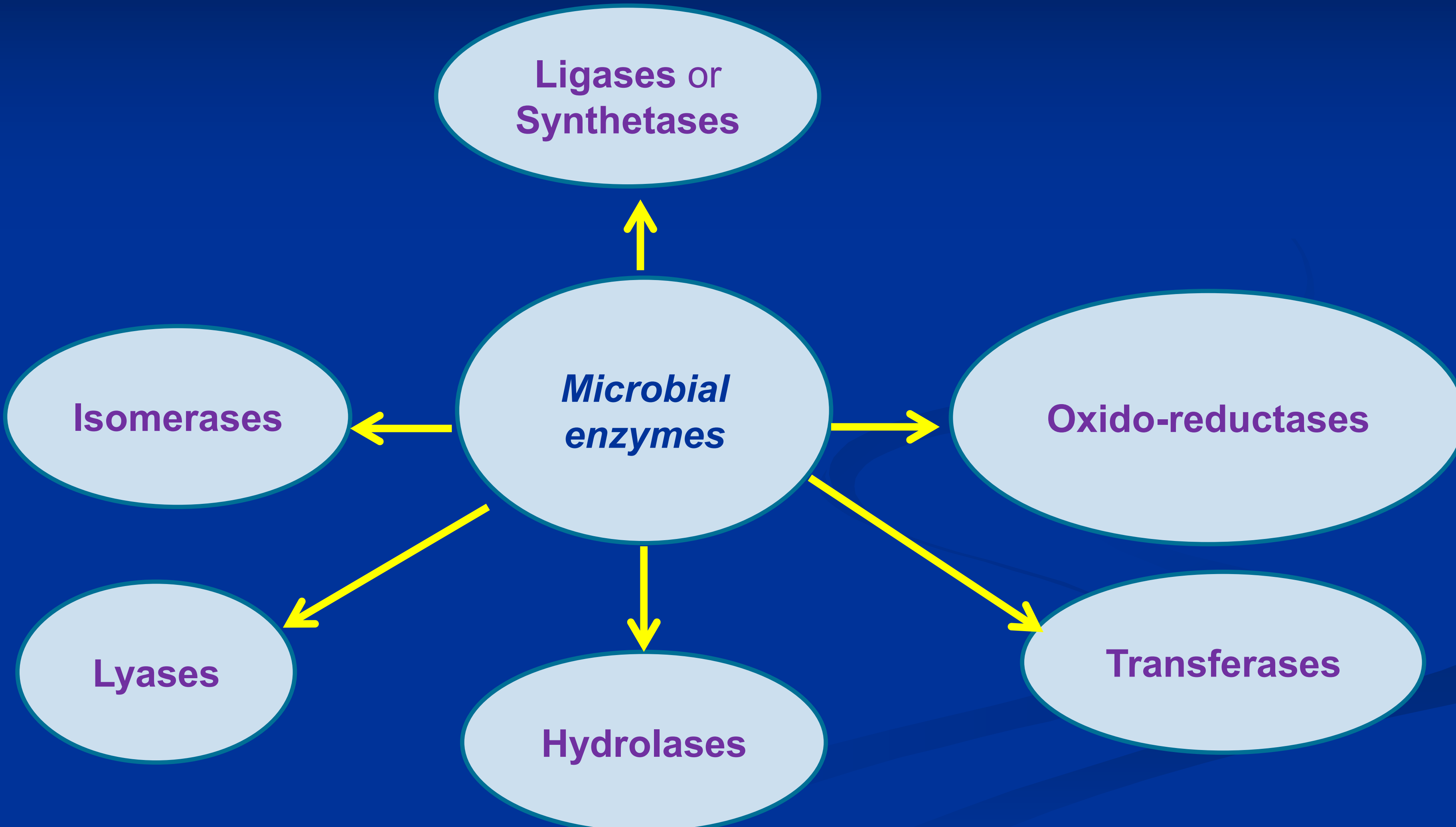
- Commercial microbial enzymes are increasingly replacing conventional chemical catalysts in many industrial processes.

- Enzymes have several advantages over chemical catalysts, including:

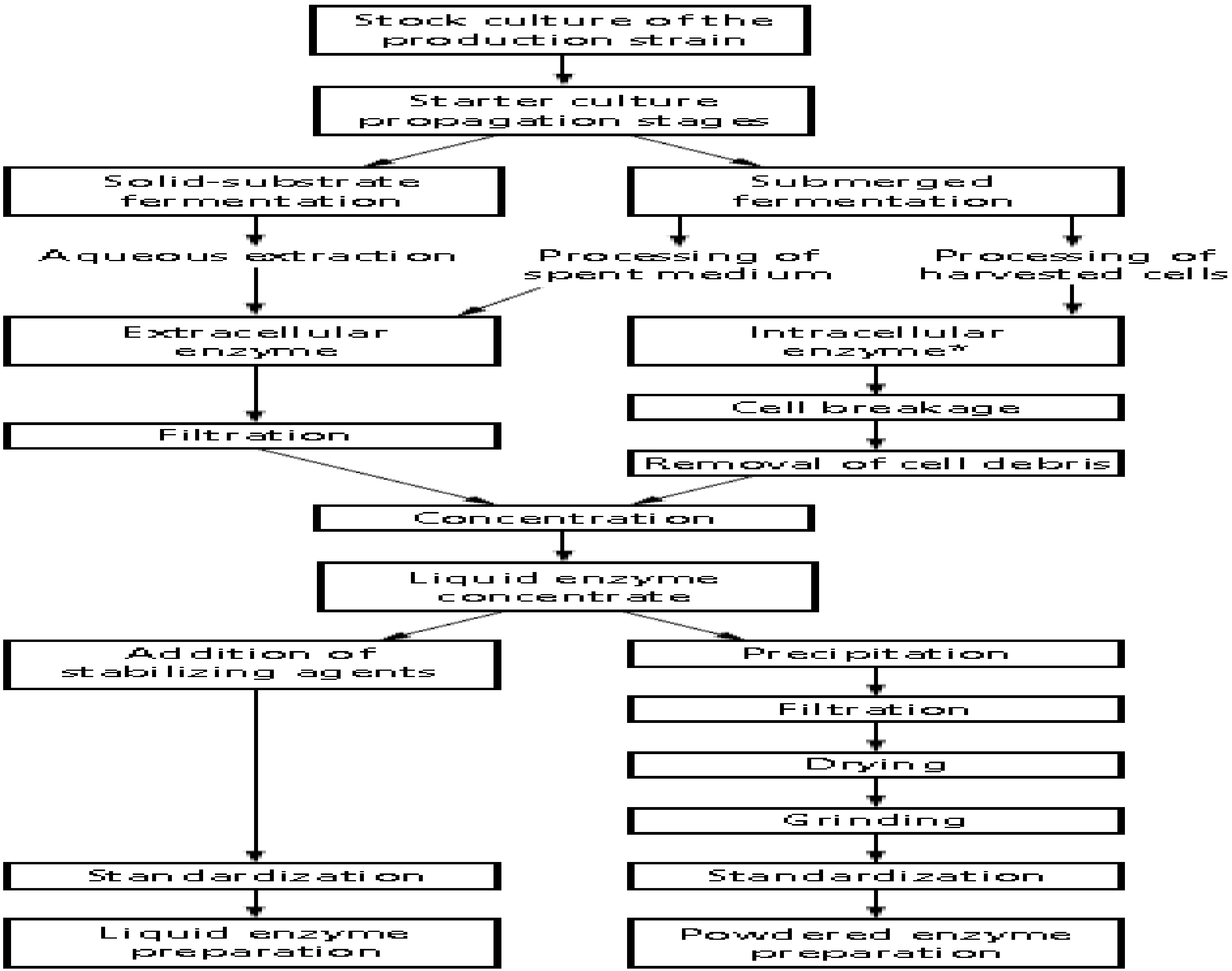
1- Enzymes are specific, often stereoselective, catalysts, which do not produce unwanted byproducts. Consequently, there is less need for extensive refining and purification of the target product.

2- Enzyme-based processes are "environmentally friendly" as enzymes are biodegradable and there are fewer associated waste disposal problems.

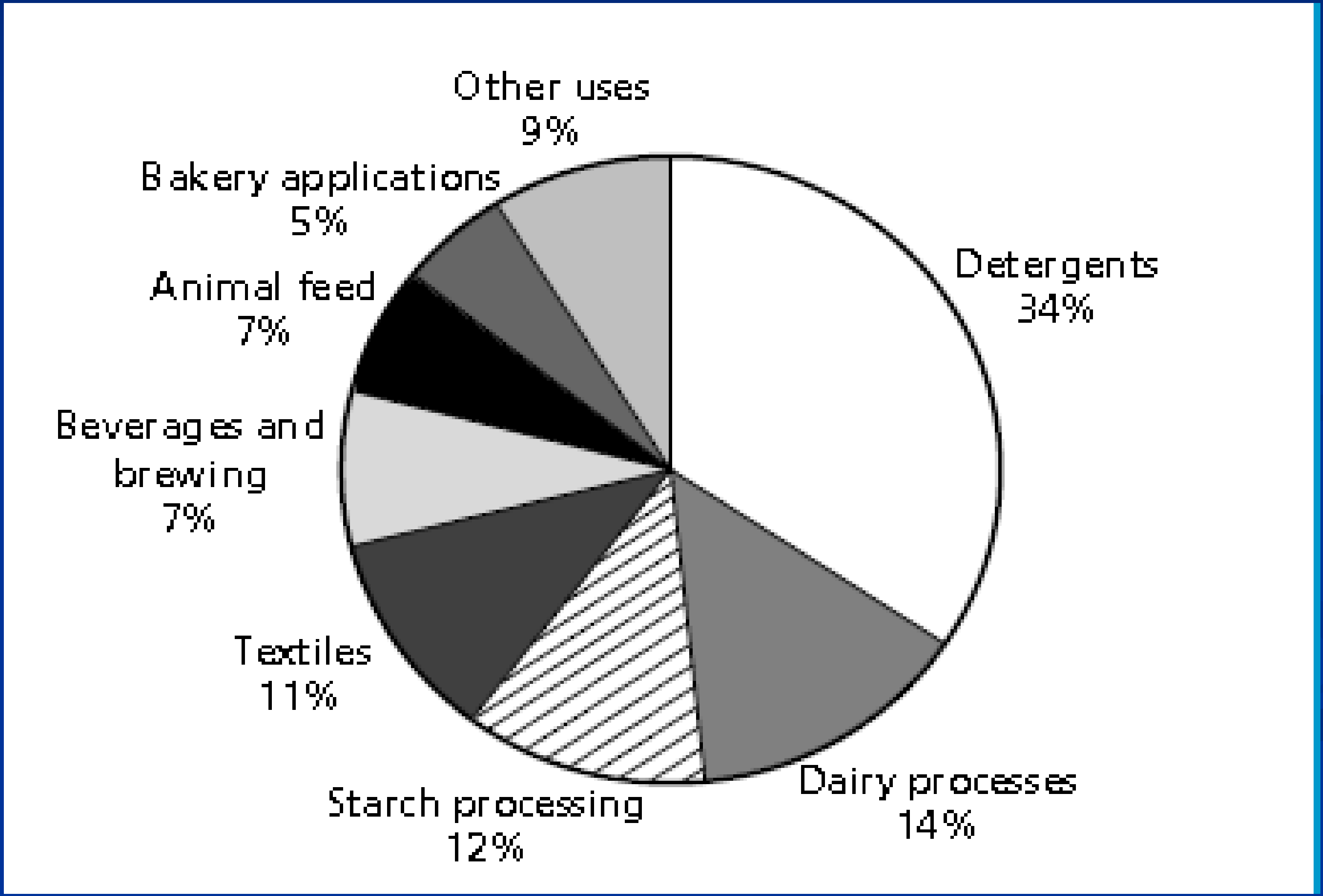
# There are six main classes, grouped according to the type of reaction catalysed



# Commercial microbial enzyme production



# Application of bulk microbial enzymes



# A. Detergent enzymes

- The incorporation of enzymes into detergents provides several benefits:

1- Energy savings are made as a lower wash temperature can be used and levels of less desirable detergent chemicals can be reduced.

2- Unlike other detergent components, enzymes do not have a negative impact on sewage treatment processes. They are totally and rapidly biodegraded to leave no harmful residues.

3- Consequently, they are environmentally safe and are no risk to aquatic life.

- These enzymes have now been engineered to improve pH and temperature characteristics, and reduce sensitivity to peroxide.

- The first commercial use of microbial enzymes in detergents was in 1959. A Swiss chemist Jaag, who worked for the detergent company Gebrüder Schnyder, developed a new product containing **a bacterial protease.**

- A well-known example is **subtilisin**, a bacterial alkaline serine protease from *Bacillus subtilis*, which is used extensively in laundry since 1960s.

- Proteases are not the only enzymes used in detergents; since the late 1980s, **amylases and lipases** have been available for incorporation.

- Superior fatty acid digesting enzymes such as **a cutinase** naturally degrades mixtures of fatty acids are also used.

# B- Starch processing enzymes and related carbohydrates

Microbial enzymes have proved to be of immense value in the processing of starch, a polysaccharide composed of amylose and amylopectin.

**1- α-Amylase:** is one of the most important of these industrial enzymes. This endo-enzyme acts randomly on α-1,4 linkages, and ultimately generates glucose, maltose and maltotriose units.

- a-Amylase mostly derived from species of Aspergillus and Bacillus.
- α-amylases are employed in several industries, mainly starch processing and baking, where starch liquefaction and dextrin hydrolysis are required.

## 2- Glucoamylase:

- From *Aspergillus niger* and *Rhizopus* species. This enzyme can completely break down starch and dextrins into glucose.

- Almost all conventional glucose production via acid hydrolysis was replaced by enzyme-based processes, because of the greater yield, higher degree of purity and easier product crystallization.

## 3- Glucose isomerase:

- This enzyme can be obtained from many bacteria, including species of *Bacillus* and *Streptomyces*, and is used in the conversion of glucose to fructose.

- The product, fructose, has the same calorific value as glucose, but its sweetening effect is approximately twice as high.

## 4- Invertase:

- This enzyme originally prepared from *S. cerevisiae* preparations, *A. niger* and *A. oryzae*.

- Used for the conversion of sucrose to glucose and fructose.

- Apart from syrup production, it is employed in confectionery manufacture. For example, soft centered chocolates are prepared by taking a solid sucrose- based filling, containing some invertase, and coating with chocolate. Within 2 weeks the centre becomes converted into a fructose/glucose syrup.

## 5- Lactase:

- Employed in hydrolysis of lactose from milk (lactose hydrolyzed to glucose and galactose).

- Whey syrup production—hydrolysis of lactose to glucose and galactose to give greater sweetness

- Mainly performed on milk and milk products for infants who are lactose-intolerant.

- Lactose hydrolysis is also useful in the manufacture of products such as ice cream, as the low solubility of this disaccharide leads to crystal formation that may give an unpleasant sandy texture. In addition, its conversion to glucose and galactose increases the relative sweetness by about four-fold.

## C- Enzymes in textile manufacture

- Enzymes are being increasingly used in textile processing for the finishing of fabrics and garments, especially in desizing, biopolishing and denim washing.

- This has previously been accomplished by treatment with acids, alkalis and oxidizing agents that may damage the fibers.

- Consequently, enzymatic desizing, biopolishing and bio-stonewashing with **amylases or cellulases** is now widely used, as they are non-corrosive and produce no harmful effluent wastes.

# *III- Fuels and industrial chemicals*

- **Acetone, butanol** may be obtained by clostridial fermentation *(Clostridium acetobutylicum)* of a range of raw materials, including starch, molasses and hydrolysed cellulosic materials.

- Members of the genus Clostridium are anaerobic Gram-positive rods, characterized by their ability to form heat-resistant spores.

- In the fermentation, anaerobic conditions is achieved by maintaining positive pressure of CO2 filtered gas over the culture.

# Lecture Summary

- **Stages of fermentation process.**
- **Differences between primary and secondary metabolites.**
- **Examples of important products produced by fermentation.**

# Quiz

## 1- Complete the following statements:

**1) Industrial fermentations comprise both ……… and ……….. stages.**

**2) Glucoamylase, invertase and lactase are used in hydrolysis of ………,**

**………… and ……………, respectively.**

**3) Phases of secondary metabolite production are …….., …….. and ………**

**4) ……. and ……. are examples of two important microbial biomasses used in human food and can be produced by fermentation.**

**5) ………. and ………. are examples of enzymes increasingly used in textile processing for the finishing of fabrics and garments.**

# Thank you!!

**Questions??**